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Patentansökan

Mönstringsmetod för biosensorapplikationer

Patterning method for biosensor applications

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Abstract

The invention relates to a patterning method of photoluminescent conjugated polyelectrolytes, polyelectrolytes or biomolecules for biosensors, biochips or other devices aimed for the detection of biomolecular interactions. These applications involve the use of biomolecules as the recognition elements and the conjugated polyelectrolytes serves as the reporter molecule. The method comprising of the following steps: (1) Cleaning of a substrate using the appropriate method; (2) placing a patterned stamp onto the substrate; (3) removal of the stamp from the substrate; (4) applying a solution of a conjugated polyelectrolyte, polyelectrolyte, biomolecule or a complex between the polyelectrolyte and a biomolecule onto the patterned surface; (5) removal of the solution after a certain incubation time or waiting until the solution has dried. The stamp (step 2) modifies the surface energy of the surface which enables molecules in the solution (step 4) to adhere to the surface in a specific way. The patterning can be carried out as a one or few pixel setup for biosensor applications or as a many pixel array of different spots for biochips.

Field of the invention

The present invention relates to a method for patterning a biosensing layer, devices such as biosensors or biochips can be made using the method. The invention is particularly concerned with methods for detection of biomolecular interactions in materials based on photoluminescent conjugated polyelectrolytes.

Background

The development of biosensor or biochip devices capable of selectively detecting biomolecular interactions using conjugated polymers (CPs) has attracted a lot of attention in recent years. CPs such as poly(thiophene) or poly(pyrrole) can be utilized to detect many kinds of analyte/receptor interactions and thus enabeling different biosensor devices. One condition to be able to use CPs for the detection of molecules in biological samples is that they are compatible with an aqueous environment. Conjugated polyelectrolytes offer possibilities for very sensitive measurements, and may become ubiquitous for genomics and proteomics in the future, if the optical or electronic processes in these materials can be used to track biospecific interactions. One such CP has demonstrated many useful interactions together with biomolecules [WO03/096016]. It is of special interest to use these CPs on a solid support to construct biosensors that can be miniaturized. Most of the work in making arrays up to date has concerned organic light emitting devices (OLED's) which make use of thin films of polymer arranged in pixels arranged to form a display, such as a flat panel display. Patterning of OLED devices has mainly been done using standard photolithography processing. This type of processing typically involves photolithographic and etching techniques which are extensive and time consuming can in most cases not accommodate biological molecules or water soluble polymers.

Patterning of the substrate with standard photolithography is achieved by using a mask between the source of radiation and a photosensitive material, a photoresist. After this step, chemical etching of the photoresist is needed. This poses some problems with chemical compatibility of biomaterials or certain polymers. One alternative to standard photolithographic methods is soft lithography. Soft lithography refers to a number of non-photolithographic techniques which have been demonstrated for fabricating high-quality micro- and nanostructures. They are called soft lithography because in each case an elastomeric stamp is the element that defines the pattern. We use polydimethyl siloxane (PDMS) as was first extensively used in Whitesides laboratory [Xia YN, Whitesides GM; (1998) Soft lithography; *Angwandte Chemie-Int. Ed.*, **37(5)**:551-575.].

PDMS has many useful properties that can be used in the formation of high-quality and high-definition patterns and structures. Soft lithography patterning techniques are based on physical contact with the surface to be patterned. Due to the elasticity of PDMS it conforms to the surface topography over a large area, even when non-planar, which makes it an excellent

molding material. The desired PDMS stamp is then fabricated by curing the PDMS prepolymer against the master. This stamp is then used for soft lithography. A number of different methods for soft lithography exist: microcontact printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC), and solvent-assisted micromolding (SAMIM).

Recently a new method for patterning of OLED devices was developed [WO 2004/006291]. They used an elastomeric rubber stamp (PDMS) is used to modify the surface of the conducting polymer poly(3,4-ethylenedioxythiophene) / poly(styrenesulfonate) (PEDOT/PSS) or the insulating polymer sodium-polystyrene sulfonate (Na-PSS). Then a luminescent polymer in solution was deposited on top of this pattern and dried. The film thereby prepared was to be used in optoelectronic devices. Methods, which use standard photolithography and aggressive chemicals or polymers unreactive towards biosensing, such as PEDOT/PSS, are not attractive ways to produce biosensors. However, methods that use bioreactive polymers and that can accommodate biomolecules in water solutions would be attractive.

An alternative method to create hydrophobic/hydrophilic patterns as in the present invention is to combine soft lithography with the self-assembled monolayers formed by alkylthiols on a gold substrate [Wilhelm, T. & Wittstock, G. Generation of periodic enzyme patterns by soft lithography and activity imaging by scanning electrochemical microscopy. *Langmuir* **18**, 9485-9493 (2002).]. This gives a hydrophilic monolayer pattern with a hydrophobic background, or vice versa, and directs solutions containing the molecules of interest to different parts of the surface to selectively adhere to these regions depending on the nature of the molecule or molecule complexes. This method also controls where molecules or molecule complexes adheres by the difference in surface free energy. However, the method requires gold as substrate which is, in most cases, unwanted in the final biosensor device since most detection principles are based on photoluminescence, which is strongly quenched at metal surfaces. The chemical step involved in the transfer of the self-assembled monolayer is also unwanted since it makes to construction of the biosensor more difficult.

Soft lithography techniques provide a simple and cost-effective way to create micro- or nanometer scale structures or patterns on various surfaces. Microcontact printing (μCP)is one of the most common soft lithographic techniques and poly(dimethylsiloxane) (PDMS) is the stamping material used in the majority of μCP studies conducted up to this date. Lately other stamping materials have been considered, such as polyolefin plastomers (POPs) [Csucs, G., Kunzler, T., Feldman, K., Robin, F. & Spencer, N.D. Microcontact printing of macromolecules with submicrometer resolution by means of polyolefin stamps. *Langmuir* **19**,

6104-6109 (2003).], POP or other plastomers can be used according to the present invention. In a recent study, antibodies were printed using PDMS stamps and their ability to bind the antigen was investigated [Graber, D.J., Zieziulewicz, T.J., Lawrence, D.A., Shain, W. & Turner, J.N. Antigen binding specificity of antibodies patterned by microcontact printing. *Langmuir* **19**, 5431-5434 (2003).]. It was demonstrated that the antibodies printed retained much of their immunological activity. When constructing patterns for use in biosensors or biochip devices it is of great importance that the biological activity is retained. The present invention provides a mean to retain the biological activity since the biological molecules are not transferred from the stamp but rather in their optimal solute. Using this method to pattern biomolecules the hydrophilicity and hydrophobicity of both the stamp and the substrate does not need to be considered. Otherwise, to successfully print proteins onto various substrates there needs to be a minimum difference in contact angle between the stamp and substrate [Tan, J.L., Tien, J. & Chen, C.S. Microcontact printing of proteins on mixed self-assembled monolayers. *Langmuir* **18**, 519-523 (2002).].

Summary of the invention

Thus, there remains a need for simple and accurate methods for making biosensor and biochip devices. The object of the present invention is therefore to provide means and methods that meet these and other requirements. These requirements to create patterns for biosensors or biochips are met through the following steps: (1) A clean substrate is provided; (2) a patterned stamp is placed on the substrate; (3) after some time the stamp is removed from the substrate; (4) a solution of a photoluminescent polyelectrolytes or a complex between the polyelectrolyte and a biomolecule is applied onto the patterned surface; (5) the solution is removed after a certain incubation time or dried.

The present invention is based on the modification of a substrate or surface using soft lithographic methods, microcontact printing (μCP) in particular. Surface or substrate modification using μCP on selected areas is a process that minimizes the waste material since only this area need to be covered with the bioreactive, such as receptor and reporter molecules, material. This is useful when the sample amount is sparse or expensive. It is also possible to print over large areas.

These modified substrates or surfaces are then used to create biosensors or biochips, defined in claim 1. One such photoluminescent polyelectrolyte that can create versatile interactions

with molecules and allows detection of molecular interactions, without any labeling of the analytes or any covalent attachment of the receptors is described in WO03096016. By the use of different solvents such as water, methanol, water/methanol blends or different biomolecules the conjugated polyelectrolyte sticks to different areas on the substrate depending on whether it has been in contact with the stamp material or not (figure 1, Route A). The conjugated polyelectrolyte can also be applied on an unpatterned surface and then modified using PDMS-stamps via the μCP method. This modification of the polymer surface controls areas where biomolecules can interact with the polymer (figure 1, Route B).

In a further aspect of the invention, a biosensor device for determining selected properties of biomolecules, can be constructed by adsorbing biomolecules from solution, without any additative patterning of the surface. Other molecules, such as photoluminescent molecules, can be added to the surface with or without using the patterning method described. This method to create the biosensor or biochip device as described above.

In still another aspect of the invention the method of determining selected properties of biomolecules, by exposing the biosensor surface described above, to a target biomolecule analyte whereby the analyte and the sensor surface interact, detecting a change of a property of said biosensor in response to the interaction between the receptor and the analyte is detected using a reporter molecule; and using the detected change to determine said reporter molecule and thus detecting property of said biomolecule analyte.

Description of the drawings

Figure 1:

Step 1, A clean substrate is provided. Route A: Step 2, A patterned or non-patterned PDMS stamp is placed on the surface of the substrate for a certain amount of time. Step 3, The solution containing the conjugated photoluminescent polyelectrolyte, biomolecule or complex between the polyelectrolyte and molecule is placed on the pattern. Step 4, After incubating a certain time the solution is removed from the surface by blowing with nitrogen gas. In some aspects the solution is left on the surface until it has dried completely. Step 5, Depending on the wettability of the solution and the solute molecules a negative or positive pattern of adsorbed molecules appears on the hydrophobic/hydrophilic pattern. Route B: Step 2, Preparation of a film containing the conjugated photoluminescent polyelectrolyte, biomolecule or complex between the polyelectrolyte and molecule from solution on the substrate. Step 3, A patterned or non-patterned PDMS stamp is placed on the film on the

substrate for a certain amount of time. Step 4, The stamp is removed and a second solution containing the conjugated photoluminescent polyelectrolyte, biomolecule or complex between the polyelectrolyte and molecule is placed on the pattern. Step 5, Depending on the wettability of the solution and the solute molecules a negative or positive pattern of adsorbed molecules appears on the patterned film. These patterned surfaces, either from route A or B, can then be further patterned according to the present invention, patterned by other methods or used as they are depending on the use of the device.

Figure 2:

A: The material is transferred from a patterned stamp to a substrate by a stamping procedure. B: A patterned stamp is used to emboss the material. C: The material is placed inside the crevices of the stamp and then transferred to the substrate. D: A patterned stamp is placed on the substrate. The material is then drawn into the channels of the stamp by capillary action; later the stamp can be removed. E: A patterned stamp is placed on the material to be patterned. Then a solvent for the material is flown into the channels of the stamp, later the stamp can be removed.

Figure 3:

A diagram showing the different steps involved in biorecognition. Receptor, reporter or receptor/reporter complex is patterned according to the present invention.

Figure 4:

The fluorescence images of patterned POWT (0,5 mg/ml) from water solution (left) and patterned POWT (0,5 mg/ml) in phosphate buffer solution (20 mM, pH 7) (right). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas.

Figure 5:

The fluorescence images of patterned POWT (0,5 mg/ml) from water/methanol (20/80) solution (left) and patterned POWT (0,5 mg/ml) in phosphate buffer (20 mM, pH 7)/methanol (20/80) solution (right). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas.

Figure 6:

The fluorescence images of patterned POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) in phosphate buffer (20 mM, pH 7) solution (top left and right). Patterned POWT (0,2 mg/ml)/double stranded DNA (2/1 on monomer basis) in phosphate buffer (20 mM, pH 7) solution (bottom left). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas. In the other images the squares are hydrophilic areas and the surrounding areas hydrophobic.

Figure 7:

The fluorescence images of patterned POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) in phosphate buffer (20 mM, pH 7)/methanol (30/70) solution (top left and right). Patterned POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) in water/methanol (70/30) solution (middle left and right). Patterned POWT (0,2 mg/ml)double stranded DNA (2/1 on monomer basis) in water/methanol (30/70) solution (bottom left). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas. In the other images the squares are hydrophilic areas and the surrounding areas hydrophobic.

Figure 8:

The fluorescence images of patterned POWT (0,5 mg/ml)/poly-glutamic acid (0,5 mg/ml) in phosphate buffer (20 mM, pH 7) solution (left) and patterned POWT (0,5 mg/ml)/poly-lysine (0,5 mg/ml) in phosphate buffer (20 mM, pH 7) solution (right). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas.

Figure 9:

The fluorescence images of patterned POWT (0,5 mg/ml)/poly-glutamic acid (0,5 mg/ml) in phosphate buffer (20 mM, pH 7)/methanol (30/70) solution (left) and patterned POWT (0,5 mg/ml)/poly-lysine (0,5 mg/ml) in phosphate buffer (20 mM, pH 7)/methanol (30/70) solution (right). In the images, thin lines correspond to hydrophilic areas and the wide lines correspond to hydrophobic areas.

Figure 10:

The fluorescence image of POWT (0,5 mg/ml) from water solution (left) after a PDMS stamp had been placed on the uniform layer to modify the said layer, no pattern is seen. After a drop containing single stranded DNA (5 nmol/ml) a pattern appears from the patterning step

(right). In the images, thin lines correspond to the unmodified areas and the wide lines are modiefied by the stamp.

Detailed description of the invention

In general terms, the present invention relates to a novel patterning method for biosensors, biochips or any other devices aimed for the detection of biomolecular interactions. The device is constructed using a few simple steps and the molecules provided for the construction can be in the preferred solution. It also relates to a patterning method for biomolecules in general terms.

The invention is based on surface modification of the surface free energy of a substrate. This modification is achieved without involving any chemical steps and can thus be achieved on a great variety of different substrates. The patterned surface is then used to bind the molecules for biomolecular recognition and detection. This step does not require covalent bonding and can be based on hydrogen bonding, electrostatic and non-polar interactions between the conjugated polyelectrolytes and the receptor molecules, herein referred to as non-covalent bonding, which further includes any type of bonding that is not covalent in its nature.

Therefore, the present invention provides a simplified, accurate and efficient method for creation of patterns for use in such devices. The need for simplified methods for construction of biosensors, biochips or any other devices aimed for the detection of biomolecular interactions is great.

A first aspect of the present invention, a method for patterning biosensors, biochips or any other devices aimed for the detection of biomolecular interactions using a patterned stamp is provided.

The method comprises of the following steps:

(1) A solid support, a substrate, including, but not limited to silicon wafers, glass (e. g. glass slides, glass beads, glass wafers etc.), polystyrene, polyethylene, gold, indium tin oxide (ITO coated materials, e. g. glass or plastics), is provided;

(2) The patterned stamp, which induces a modification in terms of surface free energy, is brought onto the surface of the substrate;

(3) After incubating the stamp a sufficiently long time the stamp is removed from the substrate;

(4) A solution of molecules, biomolecules, polymers or complexes between two or more of these is applied to area where the stamp has modified the surface. The surface

energy of the substrate in combination with the solvent and the nature of the molecules determines the deposition pattern of the biosensor layer;

(5) After a definite incubation time the solution is removed or has dried up and the pattern has been created.

A convenient way to create patterns to build microstructures for application in biosensors, biochips or any other devices aimed for the detection of biomolecular interactions using methods such as spin coating or dip coating is provided. Such microstructures may be arranged in pixels in micro feature size, narrow lines or in larger area structures depending on the need.

Standard soft lithographic methods, such as μCP or imprinting, bulk material is transferred from or to the patterned stamp or it can be used to create indentations in a surface layer in steps corresponding to (2) and (3). The work of Whitesides group is disclosed in US patent No. 5 512 131, titled "Formation of microstamped patterns on surfaces and derivative articles" (Kumar & Whitesides). This prior art disclose a process wherein a molecule capable of forming a self-assembled monolayer is coated onto the surface of an elastomeric stamp, said molecule has a functional group selected to bind to a particular material. The surface of the elastomeric stamp is placed onto the surface of the material surface and then removed to leave a self-assembled monolayer of the species according to the pattern of the elastomeric stamp.

A number of prior arts for different techniques for patterning surfaces or materials deposited thereon without using conventional photolithography are known.

An example of prior art a paper by Zhang *et al.* [Zhang, L. G.; Liu, J. F. and Lu, Z. H. Microfabrication on polymer with a contact procedure. *Supramolecular Science*, 5:713-715 (1998)] discloses the fabrication of thickness-contrast micropatterns based on a contact procedure. An array of PDMS microposts are constructed with grids which acts as the masters. This is also a contact procedure and the thickness-contrast micropatterns on the polymer can be replicated to other substrates, such as silicon wafers, with microcontact printing.

These techniques refer to soft lithography techniques and are based on material transfer by a soft rubber stamp in direct contact with the surfaces and materials to be patterned. The prior art WO0070406 (Tobias) describes a method in which a substrate coated with a material are patterned by using an elastomeric PDMS stamp by lift-off when the stamp is removed. WO0104938 (Imprinting) is a prior art describing the patterning of a material by causing indentations in the said material using a stamp. The prior art WO 2004/006291 (Xiangjun) discloses a process wherein a substrate is coated with either PEDOT/PSS or Na-PSS is

patterned by an elastomeric PDMS stamp. Said stamp does not transfer any material rather it modifies the surface of the PEDOT/PSS or Na-PSS and creates a hydrophofic/hydrophilic pattern. This pattern is later used to create displays or other optoelectronic devices.

No bulk material is transferred from or to the surface of the patterned stamp in step (2) and (3) of the method according to the first aspect of the present invention. The method disclosed in the present invention does not require any other material, such as PEDOT/PSS or Na-PSS, on the surface of the substrate and no imprinting or lift-off is done. When constructing biosensors it is an advantage if as little interfering materials is transferred as possible. Consequently the patterned stamp, in the present method, will define a pattern for a biosensing layer and will not contaminate the said layer or remove any molecules from the surface. Another aspect is that the stamp will not be contaminated during use and can thus be reused. Thus the surface of the substrate to which the molecules will be transferred in later steps is not contaminated during the patterning. A further advantage is that the patterned stamp can be used again without cleaning it first.

The patterned stamp, without any addition of material on the stamp, is contacted with the substrate in the method according to the present invention. This is in contrast with most soft lithographic methods, where the stamp is first covered/exposed to the material to be placed onto the substrate. In most cases the device material is always transferred from the stamp to the substrate during contact. However, in the present invention no layer of foreign material is on the stamp during contact to the substrate and thus no bulk transfer of foreign material to the substrate occurs. This removes the problem with depositing biomolecules or active biosensing materials on the stamp, then to transfer them onto a substrate. According to the first aspect of the present invention these materials, in the optimal solution conditions, are transferred to the substrate after it has been modified.

In particular the present invention introduces a new approach to realize devices aimed for, but is not limited to, detection of biospecific recognition through DNA (base pairing), proteins (antigen/antibody), glycoproteins or shorter purpose designed peptides. For this purpose a patterned surface of receptors and reporter molecules, such as conjugated polyelectrolytes, that can report back the interaction that has occurred between the analytes and receptors. A complex between the reporter molecule and receptors is suitably implemented in the biosensor device by using the immobilization method described. The reporter and receptor molecule can be adsorbed together or sequentially to the surface using many different methods, but not limited to, casting, dip coating, spin-coating, contact printing, screen

Contact printing is the soft lithographic technique that is particular useful for surface modification of the biosensor surface in the present invention. μCP is a straightforward method for surface or substrate modification but also for pattern generation. In the present invention the PDMS stamp is placed in conformal contact with the substrate or surface. μCP has the advantage of simplicity, accuracy and that it is reusable: Once the stamp is available, multiple copies of the pattern can be produced.

II. Fabrication of stamp templates, stamps and preparation of substrates to be patterned

The templates for the stamps can be prepared by photolithography using, but not limited to, the negative photoresist SU-8 (Micro Chem Inc., Newton, MA, USA) as the structural element on top of silicon wafers. The height of structures is chosen with respect to the dimension of the pattern but usually resides between 2-100 μm in height, and the substrate was a silicon wafer cleaned in a boiling aqueous solution containing 5% each of ammonia and H_2O_2 (TL-1 wash). The geometry for templates was designed using CleWin Version 2.51 (WieWeb Software), and transferred to a Cr mask, which was used in the photolithography step. After developing the SU-8 structures on the silicon wafer, the template, silanization (dimethyl-dicholorosilane) was done to obtain the proper surface energy of the SU-8 template. Sylgard 184 (Dow Corning, UK), a two component silicone rubber (poly(dimethylsiloxane), PDMS), was used for preparing elastomer stamps, later used for patterning the substrates or molecule coated substrates. The prepolymer and the curing agent are mixed according to the instructions provided by the manufacturer. This is then poured on templates and curing is accomplished by heating up to 130°C for at least 20 min, but lower temperatures and longer incubation times can also be used depending requirements of stamp softness. Other stamp materials can also be used in the patterning step.

The substrates to be patterned can be, but not limited to, silicon wafers, glass (e. g. glass slides, glass beads, glass wafers etc.), polystyrene, polyethylene, gold, indium tin oxide (ITO coated materials, e. g. glass or plastics) is first cleaned using the TL-1 procedure. Stamps are cut out in appropriate sizes after curing.

III. Reporter molecules

The present invention relates to a variety of reporter molecules derived from conjugated polyelectrolytes, with a minimum of 5 mers, consisting of mers derived from the monomers thiophene, pyrrole, aniline, furan, phenylene, vinylene or their substituted forms, forming homopolymers and copolymers there from. Furthermore, monomers with anionic-, cationic or

zwitterionic side chain functionalities are included within the scope of the invention. The side chain functionalities is derived from, but not limited to, amino acids, amino acid derivatives, neurotransmittors, monosaccharides, nucleic acids, or combinations and chemically modified derivatives thereof. The conjugated polyelectrolytes of the present invention may contain a single side chain functionality or may comprise two or more different side chain functionalities. The functional groups of the conjugated polyelectrolytes, charged anionic or cationic at different pH levels, make these polyelectrolyte derivatives suitable for forming strong polyelectrolyte complexes with negatively or positively charged oligomers and polymers. In addition, the ionic groups create versatile hydrogen bonding patterns with different molecules. One reporter molecule of particular interest is the zwitterionic conjugated polythiophene described in WO03/096016.

IV. Receptor molecules

The molecule that constitutes the recognition element of the present invention acts as a receptor molecule, shown in figure 3. Receptor molecules act as the recognition site for analytes through a lock and key mechanism or as anchors for performing enzymatic reactions, such as phosphorylation, the analyte in figure 3. Many different kinds of receptor molecules can be used and the choice of molecule is only limited by the ability to form a complex with the reporter molecule, to adsorb to the surface of the substrate or detector molecule and the recognition properties of desirable analytes. Appropriate receptor molecules include, but are not limited to, peptides, carbohydrates, nucleic acids (DNA, RNA, mRNA, cDNA, etc), lipids, pharmaceuticals, antigens, antibodies, proteins, any organic polymers or combination of these molecules that are capable of interacting with analytes of interest. The receptor molecules can be chemically modified to interact with the surface of the substrate or any other molecule on the substrate.

V. Analytes

Analyte molecules are such molecule that interacts with the receptor molecules in a specific way, which is the principle of the biosensor function. Appropriate analytes include, but are not limited to, cells, viruses, bacteria, spores, microorganisms, peptides, carbohydrates, nucleic acids (DNA, RNA, mRNA, cDNA, etc), lipids, pharmaceuticals, antigens, antibodies, proteins, enzymes, toxins, any organic polymers or combination of these molecules that interacts with receptors of interest. The analyte molecules can be chemically modified to interact with the surface of the substrate or any other molecule on the substrate.

VI. Immobilization on substrate

The reporter molecules, the receptor molecules or combinations thereof can be immobilized on a variety of substrate, including, but not limited to silicon wafers, glass (e. g. glass slides, glass beads, glass wafers etc., silicon rubber, polystyrene, polyethylene, teflon, silica gel beads, gold, indium tin oxide (ITO coated materials, e. g. glass or plastics), filter paper (e. g. nylon, cellulose and nitrocellulose), standard copy paper or variants thereof and separation media or other chromatographic media.

Immobilization of the conjugated polyelectrolytes is achieved by physical adhesion to the solid support according to the present invention or by entrapment in a hydrogel matrix, patterned or non-patterned.

When the receptor molecules are immobilized on the chosen solid support underneath, on top of or together with the reporter molecule of the present invention they form a complex with the polyelectrolyte through non- covalent interactions (Figure 1 and 3). This complex is formed without covalent chemistry and is based on hydrogen bonding, electrostatic-and non-polar interactions between the conjugated polyelectrolyte and the biomolecule.

Immobilization of the receptor molecules (can be biomolecules) to the reporter molecules (can be photoluminescent conjugated polymers) of the present invention may be desired to improve their ease of use, assembly into devices (e. g. arrays or parallel lines), stability, robustness, fluorescent response, to fit into the process of high-throughput-screening (HTS) using microtiter plates and other desired properties.

The conjugated polyelectrolyte and the biomolecules can be entrapped inside polymer matrices on top of a solid support or free floating in solution. A gel or network of the conjugated polymers can be formed, where each conjugated polyelectrolyte chain of the present invention is in (indirect) contact with all chains in the network. Realization of these polymer matrices can be done by mixing the conjugated polyelectrolyte with other polymers such as, but not limited to, poly (3,4-ethylenedioxythiophene)/poly (styrenesulfonicacid) (PEDOT/PSS), poly (diallyldimethylammonium chloride) (PDADMAC), poly-4-vinylpyridine (PVPy), poly (pyrrole) (PPy), poly (vinylalcohol) (PVA), poly (aniline) (PANI) or combinations thereof. By swelling these polymer blends in water a hydrogel is realized, which can be of interest when using biomolecules of biological origin. The conjugated polyelectrolytes of the present invention can be mixed together with these polymers before immobilization to the solid support or transferred afterwards. Biomolecules of interest can be

transferred together with the conjugated polyelectrolyte or in subsequent steps. Various micro or parallel line format can be used if desired, necessary or for other reasons.

VII. Arrays or lines

According to the present invention generation of large arrays or parallel lines of many different, similar or equal biosensor spots can be achieved. Arrays or lines can be used to increase ease of use, massive parallelism or other required characteristics or qualities. By using this approach many different, similar or equal analytes can be analyzed simultaneously with respect to one or different receptors. A microarray, where many individual detector elements (or probes) are integrated on a small surface area, allows massive parallelism in the detection. Since we can construct each individual detector by simply adding the molecules from solution onto a patterned surface we have reduced the number of chemical steps to a minimum for making each one of many thousands of detectors in a detector array (microarray). By using a standard micro dispenser, ink-jet printer, microfluidic network, BiaCore or other techniques a multipixel array can be prepared.

Experimental

To build efficient biosensors, biochips or any other devices aimed for the detection of biomolecular interactions efficient means for easy patterning and surface immobilization of molecules involved in the device are needed. The present invention presents a solution to these needs by demonstrating how relevant reporter molecules, receptor biomolecules or complexes of reporters and receptors can be patterned. When molecules solvated in different solvents or solvent blends are incubated on the patterned surface they adhere to different parts on the surface depending on the surface free energy. Furthermore, by the use of buffers at various pH the coating of different areas of the patterned surface can be achieved. Buffered solutions are an important aspect of the invention since the receptor biomolecules are expected to need and change properties with respect to the buffer and the pH. Solutions of receptor biomolecules or complexes between reporter molecules and receptor biomolecules can be applied onto the patterned substrate which creates a suitable patterned surface for biosensors or biochips. An important class, but not limited to, of reporter molecules is the zwitterionic conjugated polymers described in WO03/096016. This class of reporter molecules shows all the desired properties for constructing the biosensors, biochips or any other devices aimed for the detection of biomolecular interactions described in the present

invention. An important aspect of the present invention is that the reporters, receptor molecules or complexes between these can be applied to a non-patterned and then patterned afterwards according to the methods described.

Example 1: Immobilization of a zwitterionic conjugated polyelectrolyte from water solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing 0.5 mg/ml POWT in de-ionized water or in phosphate buffer (20 mM, pH 7) was then prepared and incubated for 30 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 4.

Example 2: Immobilization of a zwitterionic conjugated polyelectrolyte from a water/methanol solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing 0.5 mg/ml POWT in de-ionized water/methanol (20/80) or in phosphate buffer (20 mM, pH 7)/methanol (20/80) was prepared and incubated for 30 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 5.

Example 3: Immobilization of a zwitterionic conjugated polyelectrolyte /DNA complex from water solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) complex in phosphate buffer (20 mM, pH 7) and POWT (0,2 mg/ml)/double stranded DNA (2/1 on monomer basis) complex in phosphate buffer (20 mM, pH 7) was then prepared and incubated for 5 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 6.

Example 4: Immobilization of a zwitterionic conjugated polyelectrolyte /DNA complex from a water/methanol solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) complex in phosphate buffer (20 mM, pH 7)/methanol (30/70), POWT (0,2 mg/ml)/single stranded DNA (2/1 on monomer basis) complex in water/methanol (30/70) and POWT (0,1 mg/ml)/double stranded DNA (2/1 on monomer basis) complex in water/methanol (30/70) was then prepared and incubated for 5 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 7.

Example 5: Immobilization of a zwitterionic conjugated polyelectrolyte /peptide complex from a buffered water solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing POWT (0,5 mg/ml)/poly-glutamic acid (0,5 mg/ml) in phosphate buffer (20 mM, pH 7) solution and POWT (0,5 mg/ml)/poly-lysine (0,5 mg/ml) in phosphate buffer (20 mM, pH 7) was then prepared and incubated for 5 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 8.

Example 6: Immobilization of a zwitterionic conjugated polyelectrolyte /peptide complex from a water/methanol solution.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. Stock solutions containing POWT (0,5 mg/ml)/poly-glutamic acid (0,5 mg/ml) in phosphate buffer (20 mM, pH 7)/methanol (30/70) solution and POWT (0,5 mg/ml)/poly-lysine (0,5 mg/ml) in phosphate buffer (20 mM, pH 7)/methanol (30/70) was then prepared and incubated for 5 minutes. 30 µl of the solutions was placed on individual patterned surfaces. After 20 minutes of incubation at ambient conditions the individual droplets were removed by blowing with nitrogen gas until the substrates is completely dry. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 9.

Example 7: Modification of and transfer of DNA to a zwitterionic conjugated polyelectrolyte.

Stamps and substrates are provided according to VI in the detailed description of the present invention. The surface of the individual substrates is patterned, with respect to surface free energy, by placing separate stamps onto them and incubating for 30 min. A stock solution containing POWT (0,5 mg/ml) in water solution was then prepared and incubated for 30 minutes. 50 µl of the solution was placed on a clean glass surface. After 20 minutes of incubation at ambient conditions the droplet were removed by blowing with nitrogen gas until the substrate is completely dry. Then a patterned PDMS stamp was placed on the uniform POWT layer and a picture was taken. After this step, a drop containing single stranded DNA (5 nmol/ml) was placed on the modified POWT and incubated for 20 min and then removed by blowing with nitrogen gas. The fluorescence was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR). A 470/40nm bandpass filter for selecting excitation wavelengths and a 515 nm long pass filter for detection (exposure time: 3000 ms). The result of this patterning procedure is shown in figure 10.

CLAIMS

1. A method for creating a patterned device for biosensor applications. The method involves the following steps: (1) Providing a clean substrate; (2) placing a patterned stamp onto substrate; (3) removal of the stamp from the substrate; (4) applying a solution of a reporter molecules, receptor biomolecules or reporter/receptor complex onto the patterned surface; (5) removal of the solution after a certain incubation time or waiting until the solution has dried. The biosensor spots or pixels are created on the different areas of the patterned substrate.
2. A method according to claim 1, wherein said substrate comprises silicon wafers, glass, glass slides, glass beads, glass wafers, silicon rubber, polystyrene, polyethylene, fluorinated hydrocarbon polymers, silica gel beads, gold, indium tin oxide-coated materials, filter paper made from nylon, cellulose or nitrocellulose, standard copy paper or variants thereof and separation media or other chromatographic media.
3. A method according to claim 1, wherein the patterned stamp is a patterned elastomer.
4. A stamp according to claim 3, wherein the feature size of the pattern of the patterned stamp is in the range of from 50 nanometers to 1 millimeter or a unpatterned stamp.
5. A stamp according to claims 3 and 4, wherein in step (2) the surface energy in step (2) of any portion of the surface of the substrate that is in contact with the patterned stamp is modified.
6. A method according to any one of claims 1 to 5, wherein the topography of the surface of the substrate is unchanged after the patterned stamp has contacted the surface of the substrate and then removed.
7. A method according as claimed in claims 1 to 6, wherein the stamp is brought into contact with the surface of the substrate at appropriate humidity and temperature.
8. A method according to claim 1, wherein the reporter molecule comprises copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene, vinylene or their substituted forms.
9. The receptor molecule as claimed in claim 1, wherein said receptor molecules are selected from the group consisting of peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, organic polymers or combination of these molecules capable of interacting with said target analyte.
10. A complex between the reporter and receptor molecule as claimed in claim 1, 8 and 9.

11. A method according to any one of claims 1 to 10, wherein the reporter molecules, receptor molecules or reporter/receptor molecules is adhered to the patterned surface by transferring to said surface by a method selected from solution casting, dip coating, spin-coating, contact printing, screen printing, ink jet technologies, spraying, dispensing and microfluidic printing by the use of soft lithography, or combinations thereof.
12. A method according to any one of claims 1 to 11, wherein water, organic solvents, buffer systems or combination thereof are used as a solvent.
13. A method according to claim 1 and 2 wherein the substrate provided in step (1) is supported by one or more further device layers, where one may be a patterned device layer.
14. The device layer as claimed in 13 can be either of the reporter or receptor molecules as claimed in 8 or 9 or complexes between reporters and receptors as claimed in 10.
15. A method according to claims 1 to 14, wherein one or more further device layers on the device layer may be deposited in step (4).
16. A biosensor or biochip device for determining selected properties of biomolecules, constructed as claimed in any of claims 1-15, which said device is exposable to a target analyte.
17. The device as claimed in claim 15 and 16, wherein said target analytes are selected from the group consisting of cells, viruses, bacteria, spores, microorganisms, peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, enzymes, toxins, organic polymers or combination of these molecules that are capable of interacting with said receptors or reporter/receptor complexes.
18. The reporters, receptors or reporter/receptor complexes as claimed in claim 1, wherein said molecules are entrapped inside polymer matrices.
19. The complex as claimed in claim 18, wherein the said polymer matrices comprises poly (3,4-ethylenedioxy thiophene / poly (styrenesulfonicacid) (PEDOT/PSS), poly (diallyldimethylammonium chloride) (PDADMAC), poly-4-vinylpyridine (PVPy), poly (pyrrole) (PPy), poly (vinylalcohol) (PVA), poly (aniline) (PANI) or combinations thereof.
20. A biosensor device as claimed in claims 15 to 17, wherein said biosensor device comprises a receptacle and the polyelectrolyte is immobilized on a surface of said receptacle.
21. A biosensor device as claimed in claim 20, wherein said receptacle is a flow cell.

22. A method of determining selected properties of biomolecules, comprising: exposing the device as claimed in any of claims 1-21, to a target biomolecule analyte whereby the analyte and the receptor interact, detecting a change of a property of said reporter in response to the interaction between the receptor and the analyte; and using the detected change to determine said selected property of said biomolecule.
23. The method as claimed in claim 22, wherein the change of said property is detected by measuring fluorescence, Forester resonance energy transfer (FRET), quenching of emitted light, absorption, impedance, refraction index, mass, visco-elastic properties, thickness or other physical properties.
24. A method of manufacturing a biosensor device as claimed in claim 16, wherein the device molecules as claimed in any of claims 1-15 is attached to a substrate surface, preferably in a suitable receptacle.
25. A biosensor or biochip device, comprising a plurality of spots, arrays or lines of any of the molecules according to any of claims 1-15, immobilized on a substrate.
26. A biosensor or biochip device, wherein the plurality of spots, array or lines are printed by micro contact printing using elastomer stamps, by spotting conjugated polyelectrolyte solutions; or by ink jetting polyelectrolyte solutions onto said substrate.

Figures

Figure 1

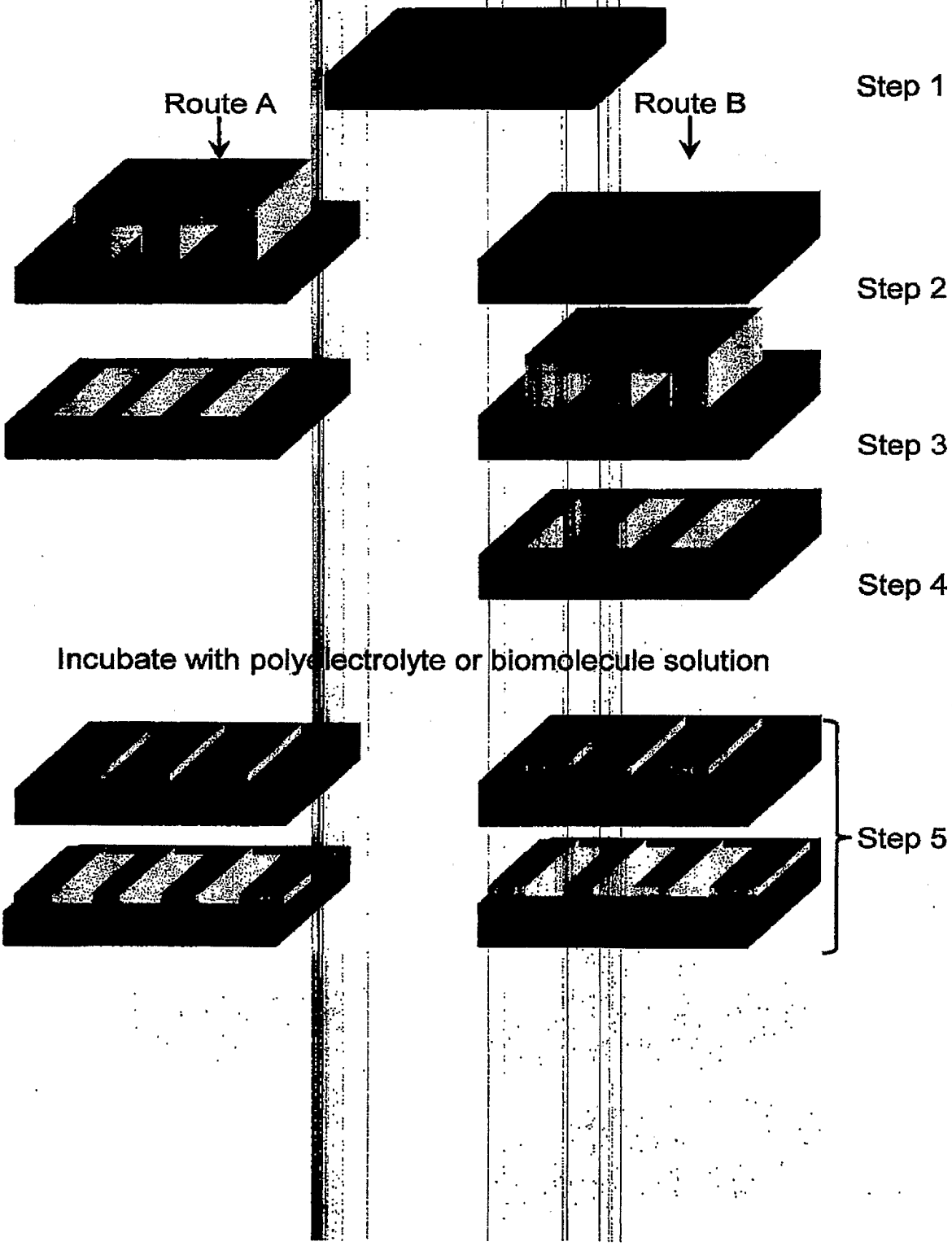


Figure 2

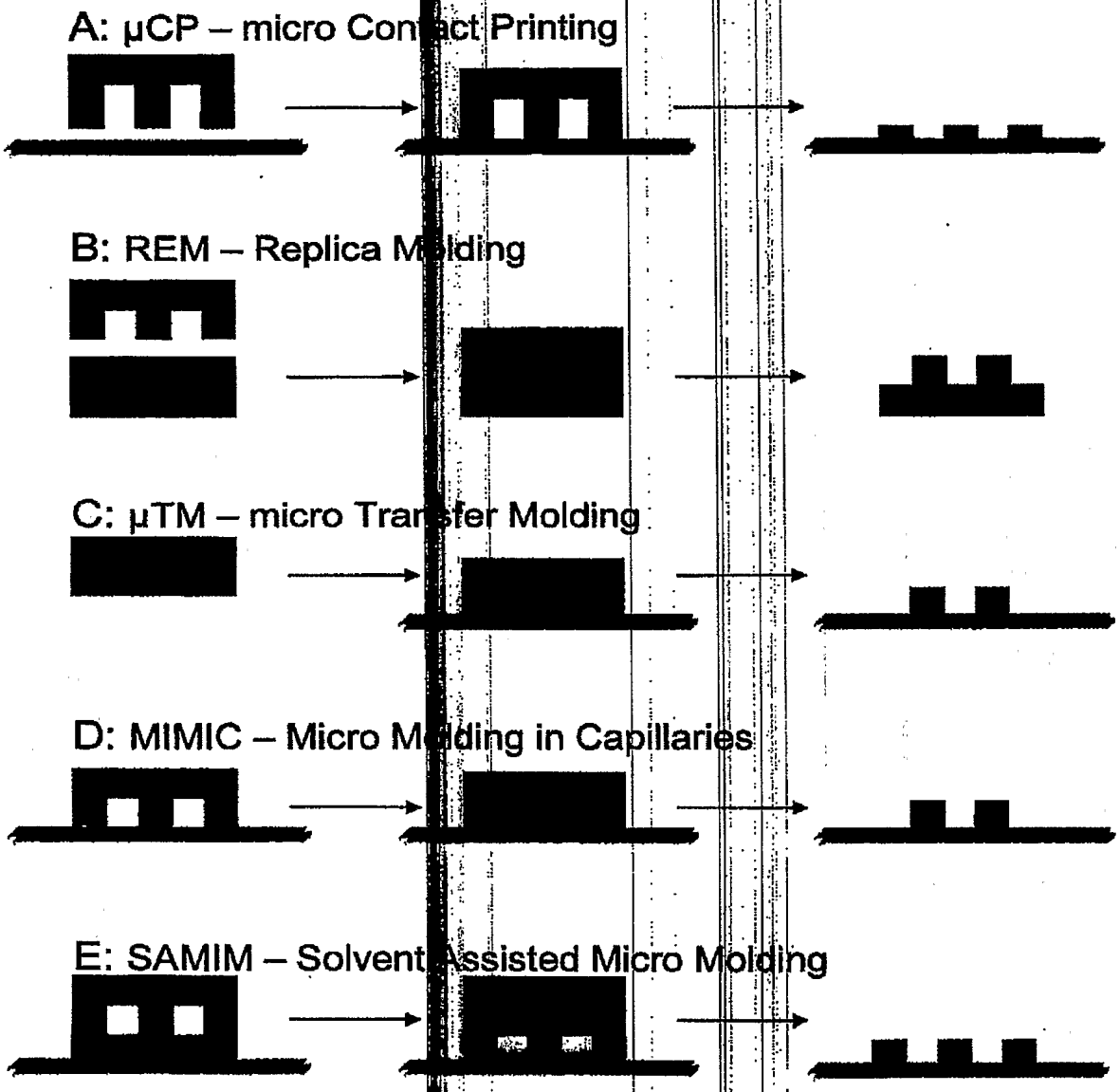


Figure 3

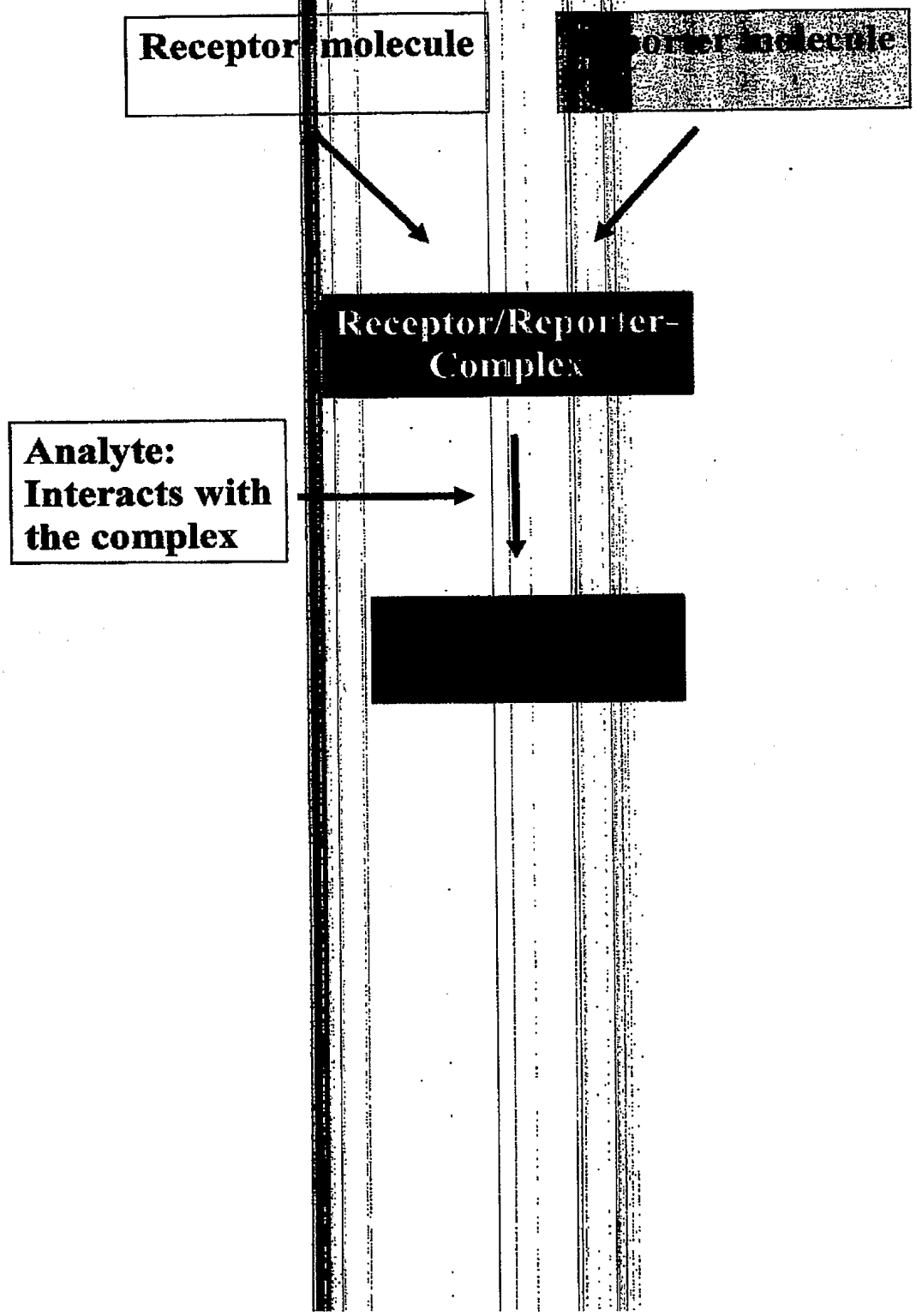
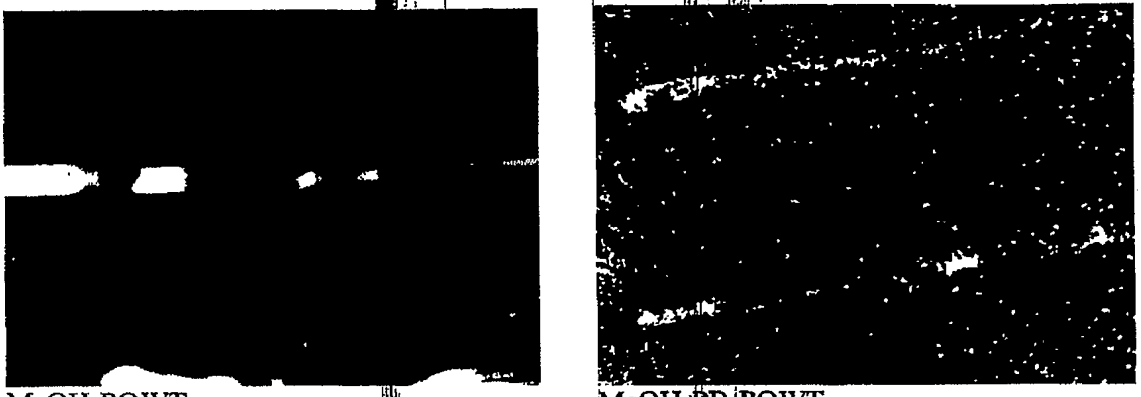


Figure 4

H2O-POWT PB-POWT

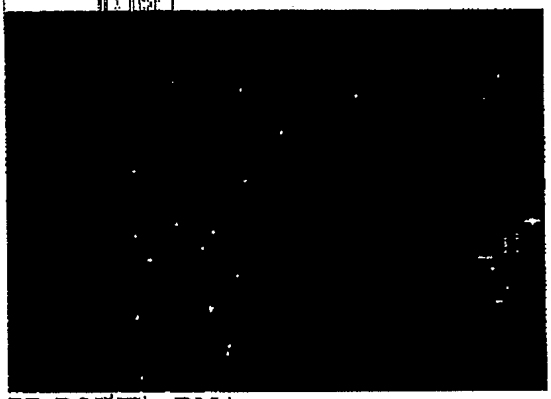
Figure 5



MeOH-POWT MeOH-PB-POWT

Figure 6

PB-POWT-ssDNA

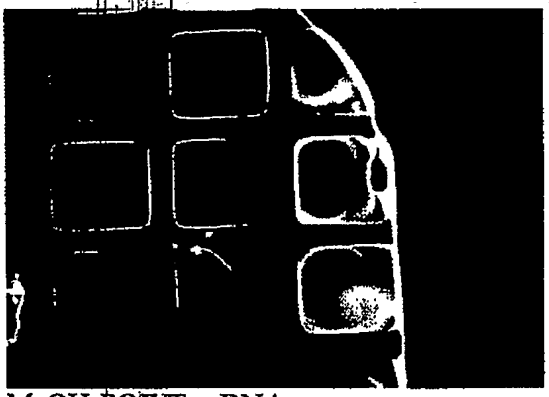


PB-POWT-ssDNA

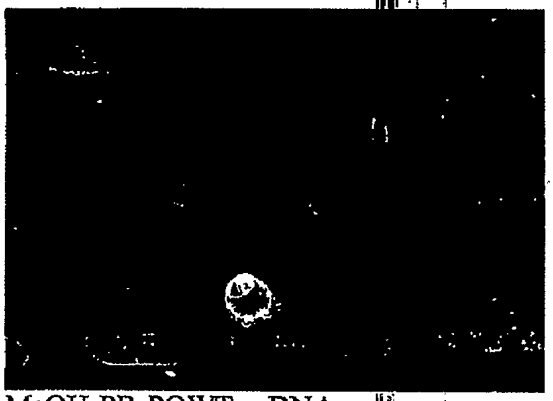
PB-POWT-dsDNA

Figure 7

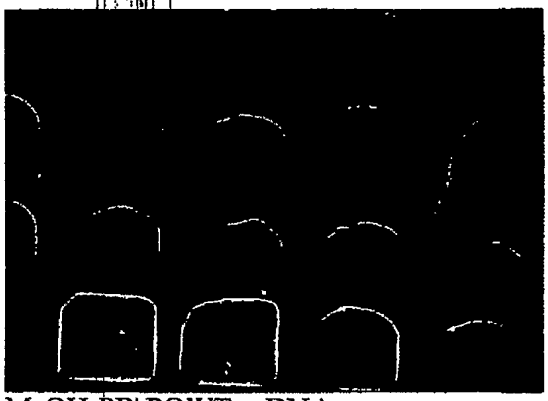
MeOH-POWT-ssDNA



MeOH-POWT-ssDNA



MeOH-PB-POWT-ssDNA



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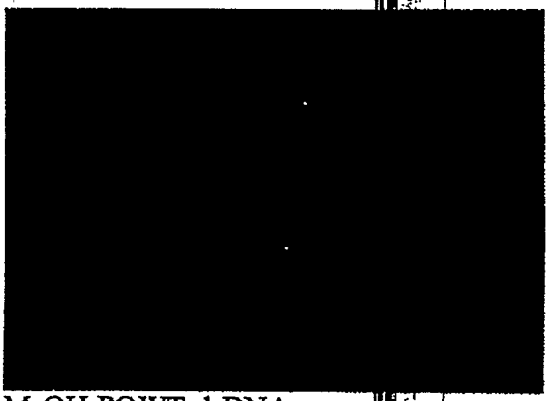
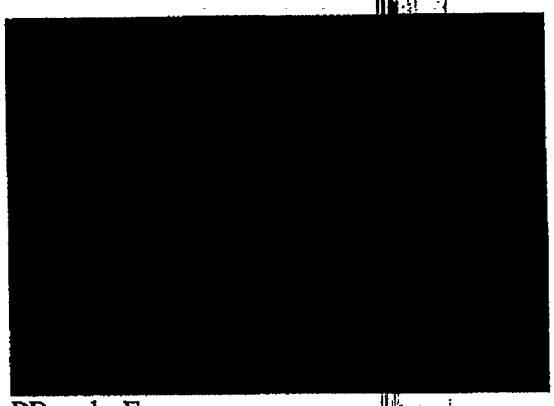
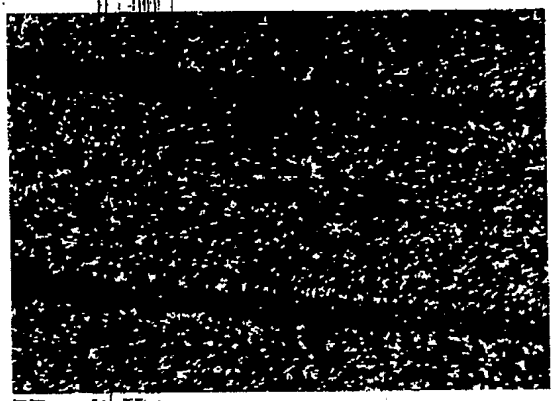


MeOH-POWT-dsDNA

Figure 8

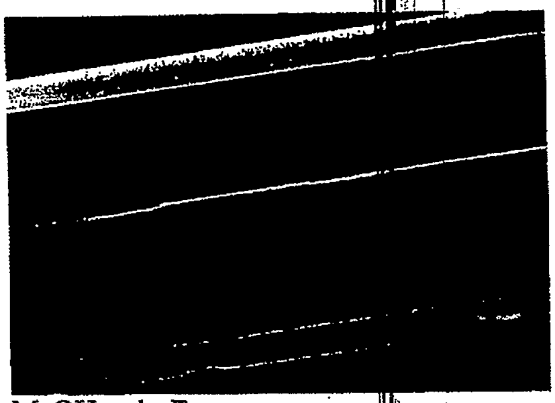


PB-poly-E

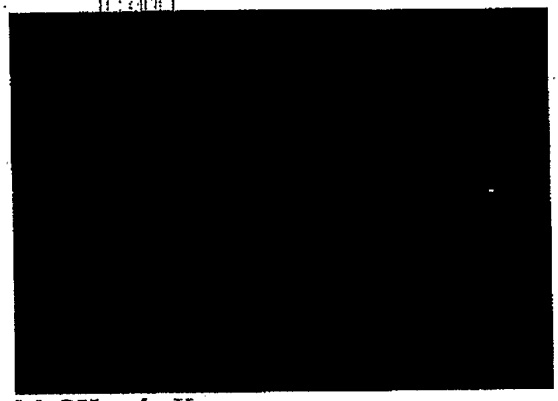


PB-poly-K

Figure 9

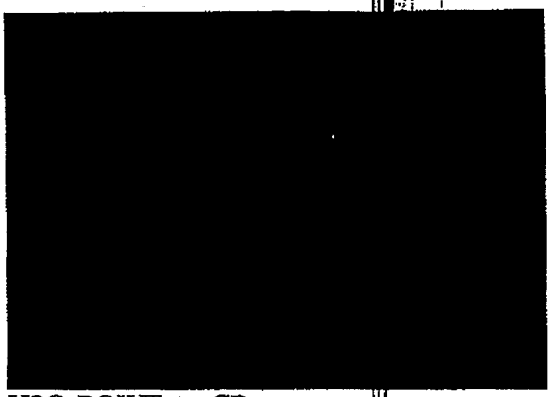


MeOH-poly-E

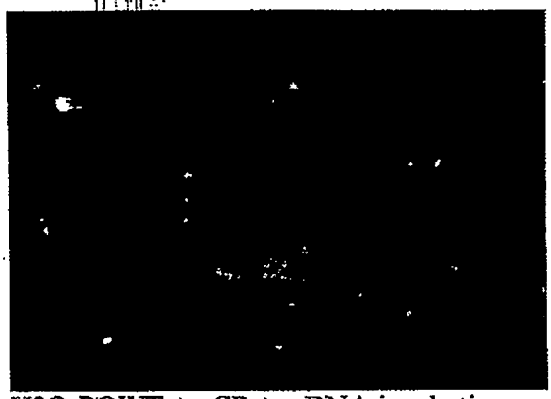


MeOH+poly-K

Figure 10



H2O-POWT + uCP



H2O-POWT + uCP + ssDNA incubation